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Effects of allochthonous dissolved organic material on the periphytic microbial communities of lake littoral

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ABSTRACT

During the last decades, increasing concentrations of terrestrially derived dissolved organic carbon compounds and, consequently, darker watercolor has been assessed in boreal lakes. This phenomenon is induced by several factors such as long-term increase in precipitation and soil runoff as well as land-use changes, e.g. enhanced forest management in the drainage area. However, the influence of increased terrestrial DOC load on the littoral microbial periphyton has been rarely studied despite the central role, which periphyton has in the transfer of energy and material in the aquatic ecosystem and especially in the littoral area. In this thesis, the main aim was to evaluate, how a permanently different DOC flow and, on the other hand, a drastic pulse of soil water influence the function and phylogenetic composition of littoral periphytic microbial communities in boreal lakes. The long-term carbon flow and microbial periphyton were studied in three lakes with permanently dissimilar concentration and quality of DOC in lake water. The results show that between these lakes, quality of both allochthonous and autochthonous carbon pool as well as microbial community composition were different in periphyton. It was also evident, that several physical, chemical, and biological factors influence structure of the periphytic carbon pool in concert. According to these results, after a long-term change in allochthonous DOC flow to recipient lake, phylogenetic shift in the periphyton microbial community will occur. However, drastic soil water pulse into littoral area seem to cause short-term enhancing and suppressing metabolic impacts to the microbial communities of surface sediment, but no shift in the community composition was found. Metabolic changes observed were not related to changes in phylogenetic community structure. Thus, periphytic microbial communities seem to be resistant against metabolic or structural shifts after drastic soil water pulse. It was also evident that the presence of macrophytes (*Lobelia dortmanna*) altered the response of sediment bacterial communities on humic compounds deposited on sediment. These results emphasize the versatility of microbial reactions, which occur in littoral periphyton after increased terrestrial carbon load.

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LIST OF ORIGINAL PUBLICATIONS

1) Heikkinen, E., Haukka, K., Björklöf, K., Sivonen, K. and Kairesalo, T. 2005. Structural and functional responses of bacteria living in rhizosphere and macrophyte free sediment to humus addition into water column. Verh. Internat. Verein. Limnol. 29: 857-860.

2) Heikkinen, E., Haukka, K., Björklöf, K., Sivonen, K. and Kairesalo, T. Metabolic and structural responses of sediment bacterial communities to humus addition into the overlying water column. (manuscript)

3) Heikkinen, E., Hartikainen, H. and Kairesalo, T. Dissolved organic carbon in lake water affects carbon chemistry and biology of epiphytic biofilms via allogenic and autogenic factors. (manuscript)

The publications are referred to in the text by their roman numerals I, II and III.

Authors contributions to the publication and manuscripts:

1) All authors participated to the designing of the study. EH was responsible for setting the experiment up and collecting the data presented in this article except CLPP analysis, which was made by KB and molecular genetic analysis, which was made by KH. EH wrote the article as the main author. All authors commented the manuscript.

2) All authors participated to the designing of the study. EH was responsible for setting the experiment up and collecting the data presented in this manuscript except CLPP analysis, which was conducted by KB and molecular genetic analysis, which was made by KH. EH wrote the manuscript as the main author. All authors commented the manuscript.

3) TK, HH and EH designed the study. EH conducted the study, made most of the analysis and collected all the data. EH wrote the manuscript as the main author. All authors commented the manuscript.

ABBREVIATIONS

DOC	dissolved organic carbon
DOM	dissolved organic matter
HMW	high molecular weight
LMW	low molecular weight
HS	humic substances
C:N:P ratio	carbon:nitrogen:phosphorus -ratio
PP	primary production
BP	bacterial production
Chl *a*	chlorophyll *a*
BCC	bacterial community composition
EPS matrix	exopolysaccharide matrix
FI	fluorescence index
UV	ultraviolet radiation (100 – 380 nm wavelength of light)

1. INTRODUCTION

1.1. Terrestrial carbon flow in the pelagial and littoral areas of boreal lakes

In the northern boreal areas, a high amount of dark-water humic and polyhumic lakes exists. The proportion of terrestrial organic carbon in the surface water of these lakes is high, even 50 – 90% of the total carbon present in lakewater (Meili 1992, Rantakari and Kortelainen 2008). These terrestrial origin carbon compounds, which mostly consist of humic substances (HS), affect in many ways the physical and chemical properties of aquatic ecosystems. They influence spectral characteristics of light and attenuate solar radiation which penetrates to the water column thus reducing the depth of photosynthetic layer and influencing thermal conditions in lake (e.g. Karlsson et al. 2009, Seekell et al. 2015). Humic substances also bind a variety of organic compounds and metals as well as have a strong influence on nutrient recycling in aquatic environments. This influence occurs as transportation of organic nitrogen compounds with dissolved organic carbon (DOC) from soil to recipient waters (Kortelainen et al. 2006a) and through complexion of DOC with Fe-oxides and phosphate ions in water column (Jackson and Schindler 1975, Shaw et al. 2000). Carbon-iron-PO_4 -complexes and colloids aggregate to larger flocs and sediment transporting nutrients to sediments (Kortelainen et al. 2004, Einola et al. 2011). Through these interactions HS have a strong effect on the living conditions and productivity of aquatic biota (e.g. Ask et al. 2009).

About half of the terrestrial carbon load which flows to inland waters finally ends up to oceans. Sedimentation and microbial as well as light-induced decomposition together respond half of the sink of terrestrial organic carbon in lakes (Cole et al. 2007). Allochthonous aquatic humic compounds mostly consist of high molecular weight complex carbon substrates, humic and fulvic acids, which have traditionally been thought to be recalcitrant against microbial degradation (e.g. McDonald et al. 2004). However, in the last decades the importance of dissolved organic matter and humic substances on supporting heterotrophic metabolism and productivity in aquatic environments has been perceived (Hessen 1985, Tranvik 1988, Tanentzap et al. 2017). Respiration of bacteria in the water column and sediment and consequent emission of carbon dioxide to the atmosphere has been assessed to be the major

pathway of organic carbon from lakes (e.g. Kortelainen et al. 2006b, Sobek et al. 2006). In humic boreal lakes, significant losses of dissolved organic carbon from the water phase occur as a consequence of sedimentation (Molot and Dillon 1997, Kortelainen et al. 2004) and sediments have been estimated to be a sink of terrestrial-origin organic carbon (Gudasz et al. 2012). Sedimentation rate of allochthonous matter has been found to correlate with the concentration of terrestrial DOC in water (von Wachenfeldt and Tranvik 2008). Thus, coagulation and flocculation of allochthonous DOC and consequent sedimentation of aggregates obviously play a significant role in the carbon budget of humic lakes.

In lakes, littoral area is the first habitat receiving terrestrial runoff, and it has a significant role in the total productivity of lake ecosystems, especially in the shallow lakes (Wetzel 2001). It has been recognized that the biodiversity of aquatic environments is highest in the transitional zone between the littoral and pelagial areas (Wetzel 1999), and that carbon fixed in the littoral area is transported to the pelagial area (Andersson and Kumblad 2006). Thus, littoral areas are enormously important in overall function of the lake ecosystems. As decomposers of auto- and allochthonous organic material heterotrophic microbes have a key function in releasing and recycling carbon compounds and other nutrients in the littoral zones, where microbial activity is enhanced due to the higher water temperature as well as higher nutrient and labile substrate concentrations as compared to the lake pelagial areas (e.g. Bergström et al. 2010, Stets and Cotner 2011). In the dark water humic lakes, which have traditionally been attributed as net-heterotrophic (e.g. Jansson et al. 2000), the importance of littoral area as related to the total lake productivity has been underestimated. However, in the recent studies the littoral periphytic primary (PP) and bacterial production (BP) have been assessed to make a strong contribution on the whole-lake PP and BP also in highly-humic boreal lakes (Vesterinen et al. 2016; 2017). According to these results, the metabolism type of a humic lake can occasionally shift from heterotrophy to autotrophy because of the high PP of the periphytic biofilms.

Periphyton living on surfaces like on the aquatic macrophytes and sediments is referred as a hotspot of microbial metabolic activity (Battin et al. 2003a). Sediment bacterial communities have a significant role in nutrient recycling in the whole aquatic ecosystems (e.g. Martins et al. 2011). Especially the uppermost layers of sediment have high microbial productivity and mineralization activity (Haglund et al. 2003), consequently exporting large quantities of

unassimilated DOC to the water column (Sinsabaugh and Findlay 1995). Overall, the sediment surface as well as epiphytic and other periphytic communities have a great importance in retention and selection of organic and inorganic substances in lakes and rivers (e.g. McDowell 1985). Both the physical structure (e.g. porosity) of periphytic biofilms and ability of molecules to diffuse into the biofilms determine the transportation rate of substances from the overlying water to the periphytic material (Battin et al. 2003b). In streams, these biofilms have been assessed to increase both hydrodynamic transient storage and retention of particles by several hundreds of percent (Battin et al. 2003b). As microbial species structure determines the metabolic effectiveness of the biofilm, periphytic bacterial community composition has a central selective role in aquatic chemistry of shallow aquatic environments.

As principally in all ecosystems, also in aquatic ecosystems microbial community composition determine the function and effectiveness of prevailing microbial processes (e.g. Martins et al. 2011). Several factors as interactions between certain phytoplankton and bacteria phylogenetic groups as well as selective grazing pressure have been assessed to influence the microbial activity and bacterial community succession in lakes (Paver et al. 2013, Staley et al. 2015). Also presence of different carbon substrates available to microbes forms the phylogenetic composition and metabolism of microbial communities in aquatic environments (Crump et al. 2003, Judd et al. 2006), although e.g. Eiler and colleagues (2003) showed evidence that in natural lake water concentrations carbon substrates are not able to determine the microbial community composition. Also, in the water column of humic lakes the presence of certain bacterial phylogenetic groups has been emphasized (Lindström and Leskinen 2002), although the presence of humic substances solely has not been assessed to have direct effect on the planktic community composition of bacteria (Lindström 2000).

Despite the importance of aquatic periphytic communities on the metabolism of lake ecosystems, studies concerning the influence of varying carbon substrates on bacteria living in littoral benthic and other periphytic habitats are rather scarce. In streams and rivers, some research about the response of periphytic microbial communities as related to e.g. concentration and quality of DOC compounds has been conducted (McNamara and Leff 2004, Gao et al. 2005). For instance, seasonal changes in quality of aquatic organic material have been found to change microbial community composition both in clear water sediments

(MacGregor et al. 2001) and boreal dark-water lake sediments (Steger et al. 2011). However, knowledge about impacts of variation in allochthonous carbon flow on aquatic littoral habitats is still limited.

1.2. Anthropogenic influence on dissolved organic matter loading from the watershed

Human activities, e.g. forestry practices, ditching and peat mining, have a strong influence both on the long term carbon and nutrient load and the drastic soil water pulses to aquatic environments from the drainage areas (Schelker et al. 2012, Palviainen et al. 2014). Natural variation in the intensity of precipitation and surface runoff as well as other phenomenon such as spring snowmelt also influence the DOC flow into watersheds (e.g. Canham et al. 2004, Dyson et al. 2011). Effects of forest management on the leaching of organic and inorganic substances from forest soil to surface waters have been studied since 1960`s (Bormann et al. 1968). Different managements such as clear-cutting and restoration of forestry-drained peatlands are found to increase dissolved and particulate carbon (C), nitrogen (N) and phosphorus (P) concentrations both in the soil water and recipient waters (Ahtiainen 1992, Rosén et al. 1996, Koskinen et al. 2017, Nieminen et al. 2017). Forest harvesting also enhances leaching of alkaline and earth alkaline cations, and other metals and affects pH and temperature of the soil water and recipient waters (e.g. Reynolds et al. 1995). These responses are mainly attributable to changes in soil hydrology, deposition of aerial ions and vegetational uptake of nutrients as well as enhanced mineralization and nitrification in the clear-cut area (e.g. Vitousek et al. 1979, Stevens and Hornung 1990).

In Finland, forestry practices have been estimated to produce 5 % of N load and 8 % of P load to aquatic environments (Finnish Government decision-in-principle on Water Protection Policy Outlines to 2015). Recently, studies have shown that these proportions can be even higher and the impact of forest practices on leaching of nutrients from the soil to recipient waters may last much longer than was expected earlier, even decades (Nieminen et al. 2017). Nowadays, it is widely recognized, that ditching and the ditch network maintenance as well as other forestry practices enhance transport of particulate and dissolved carbon compounds from forest areas to recipient waters (e.g. Winkler et al. 2009, Rodgers et al. 2010). In small forest ponds and brooks, the local impact of forestry practices may be even much stronger than expected on the

basis of average figures. In recipient aquatic environments, forestry-induced nutrient and carbon load may cause eutrophication, enhanced algal production and accumulation of organic material on the surfaces (e.g. Holopainen and Huttunen 1998). In addition to such short-term incidents enhancing terrestrial carbon and nutrient load, also large-scale variation in flow of dissolved compounds from drainage areas to surface waters currently exists.

During the last decades, increasing watercolor of lakes, the so-called browning effect or brownification, has been found to occur in large scale in boreal and northern temporal areas (e.g. Hongve et al. 2004, Monteith et al. 2007). This phenomenon is caused by higher concentrations of DOC compounds in lake water, attributable to climate change induced higher average temperatures, increased precipitation and enhanced runoff and transportation of solutes from the watershed (Freeman et al. 2001, Lepistö et al. 2014, De Wit et al. 2016). Also reduced loss of iron from the lake water due to improved pH conditions and consequent higher iron concentrations in water (Weyhenmeyer et al. 2014) as well as increased solubility of soil organic material because of declined acid deposition (Monteith et al. 2007) have been suggested as reasons for the increased watercolors. Browning influences function of the entire aquatic ecosystem through several mechanisms such as a shifting food web structure to more heterotrophic direction and through changing planktic algal community composition in lakes (Solomon et al. 2015, Taipale et al. 2016, Creed et al. 2018). Also, declined abundances of macroinvertebrates concurrently with brownification in boreal humic lakes have been assessed (Arzel et al. 2020). Increased runoff from the drainage area may also cause higher leaching rate of humic-like substances from the soil as well as higher proportion of HS of the total aquatic DOM (Kothawala et al. 2014), thus changing the quality of aquatic DOC to more recalcitrant direction. Such kind of change might have a strong influence on carbon sequestration in aquatic ecosystems through several mechanisms as increasing carbon storage to the sediments.

2. OBJECTIVES

In the thesis, my aim was to evaluate how terrestrial soil water flow and allochthonous carbon it consists affects quality of carbon pool and selected variables of microbial communities in the periphyton of littoral area. The mechanisms and influence of terrestrial DOC on periphyton in littoral areas has been rarely studied despite the central role, which biofilms has in the total

littoral metabolism. Littoral area also receives allochthonous inflow as a first habitat in the lake and the immediate and most intense biological and chemical reactions caused by fresh carbon substrates could be expected to occur in that area of the lake ecosystem. As the sedimentation of humic substances has been evaluated to be an important sink of carbon in humic lakes, I wanted to assess especially the influence of sedimentation on the periphytic microbial communities. To trace the complex habitat structure typical to the littoral areas, I also studied whether the presence of macrophytes has an influence on microbial reaction after humus addition.

By the studies fulfilled, I wanted to evaluate, how a) a long-term flow and b) a drastic pulse of soil water influences the function and phylogenetic composition of littoral microbial communities. Recent human activities have caused both large-scale increase in the aquatic DOC concentrations in lakes due to increased precipitation and abrupt humus pulses and accrual of organic material into aquatic ecosystems as a consequence of e.g. forestry practices and peat mining. Further consequences of such kind of processes need to be evaluated more accurately. As the experimental littoral periphytic communities, surface sediments in the artificial mesocosms and natural epiphyton were used.

Presentation of the papers

(I) In paper I, I assessed, whether and how the presence of macrophytes changes the sediment microbial response on drastic terrestrial soil water pulse in the artificial mesocosms. With this experiment, the role of structural versatility of littoral area on microbial response on soil water pulse was evaluated.

(II) In paper II, the metabolic and phylogenetic response of sediment surface bacteria on the short-term soil water pulse was assessed in the same experiment as presented in article I.

(III) In paper III, the influence of long-term carbon load on the quantity and quality of periphytic carbon pool as well as periphytic microbial community structure was assessed. In the study, three lakes with naturally different DOC concentration and quality as related to aromatic carbon compounds, were used as study sites.

3. MATERIALS AND METHODS

3.1. Study sites

All the field studies were done in Nastola area (61º00'N, 25º50 'E), Southern Finland. In the humus extract addition experiment (I, II), all biotic and abiotic materials were collected from Lake Ahvenlammi (61°00' N, 25°53' E), an oligotrophic lake with clear water (Secchi depth about 5.2 m and watercolor 15 mg Pt/l).

In the study concerning the deposition rate and quality of periphyton under field conditions (III), three lakes with dissimilar quantity and quality of aquatic DOC were studied. Characteristics of the lakes are shown in Table 1. A detailed description of the DOC concentrations as well as parameters describing the quality of aquatic DOC compounds in these lakes are shown in paper III (Fig. 1 A and B; Table 3).

Table 1. Characteristics of the study lakes (III)

	Lake area ha	Max.depth m	Volume M m^3	Catchment km^2	Catchment: lake area
Ahvenlampi	12	12	0.5	0.44	3.67
Sammalisto	12	6.5	0.3	2.2	18.3
Kuorelampi	9	7.8	0.2	0.85	9.44

3.2. Experimental set-up

3.2.1. Humus extract addition -experiment (I, II)

The humus addition experiment was carried out under laboratory conditions within eight steel mesocosms. Details of the structure and dimensions of the mesocosms have been described in the manuscript (II). Four of the mesocosms had humus extract (containing also organic and inorganic nutrients) added to them (referred to as humic-water mesocosms or humus treatments). The other four mesocosms were supplemented with similar amount of nitrogen and phosphorus added as inorganic form (referred to control mesocosms or control

treatments). Preparation of the humus extract has been described in the manuscript (II). The experiment was started by replacing 1/5 of the water with humus extract in the humic-water mesocosms. After the amendment, watercolor of the humic-water mesocosms increased up to 80 mg Pt/l.

Sediment and isoetid macrophytes used in the experiment were collected from the lake in June. A more detailed description of the sampling, pre-treatment and quality of the sediment set into the mesocosms is presented in the manuscript II. After adding 220 l of filtered lake water to each mesocosm, the fine organic material was allowed to settle for two weeks. Water in the mesocosms was circulated with pumps to keep the water column aerobic. The laboratory light cycle was set to 8/16-h dark/light periods and water temperature was adjusted to 15°C. The photon flux density on the surface of the shallow-water sediment was 74.5 µmoles m^{-2} s^{-1} in the humic-water mesocosms and 87.8 µmoles m^{-2} s^{-1} in the control mesocosms. The corresponding values for the deep-water sediment were 20.4 and 40.8 µmoles m^{-2} s^{-1}.

3.2.2. Deposition rate and quality of periphyton in the lakes (III)

In lakes Ahvenlampi, Sammalisto and Kuorelampi, littoral water was sampled, and material deposited in the littoral area was collected five times between May and October in three comparable locations. In each location, material was collected in thirty-six 9 cm high plastic tubes, which were placed for six days on the sediment surface (depth of 40−60 cm). Material in the tubes was combined as one sample in each location. When choosing the sampling sites, special attention was paid to the similarity of shore vegetation and the steepness of shore banks between the lakes.

3.3. Analyses

3.3.1. Humus extract addition -experiment

Surface sediment samples (0,5 cm thick surface layers) both from the shallow- and deep-water parts of the humic-water mesocosms and control mesocosms were collected on experimental days 1, 2, 4, 8, 15, 22, 36, and 50. From the sediment samples, the dry matter content, the organic matter content and carbon and nitrogen contents as well as the pH were measured.

From sediment samples the number (only on days 1, 22, and 50) and biomass production of bacteria as well as extracellular activities of microbial β-glucosidase, acid phosphatase and aminopeptidase were measured. The phylogenetic structure of the bacterial communities in the sediment was assessed by Denaturing Gradient Gel Electrophoresis (DGGE; on days 1, 22, and 50). PCR amplification for purified DNA was fulfilled using general bacterial primers F-968-GC and R-1401 targeting the 16S rRNA gene. Community-level physiological profiling (CLPP) of the sediment microbial communities was analysed by measuring the utilization of 95 carbon substrates (only on days 1, 22, and 50). CLPP measurements were done only for deep-water sediments. More detailed descriptions of the sampling schedule as well as the procedure of methods and analyses used are given in the paper II.

Table 2. Analyses of sediment bacterial features and the methods used in the mesocosm experiment (I, II).

Analysis	Method	Original reference(s)	Analyser used
Number of bacterial cells	DAPI-staining	Porter and Feig (1980)	Epifluorescence microscope Nikon E600
Bacterial biomass production in sediment	Incorp. of [14C] leucine into proteins, combustion	Tuominen and Kairesalo (1992)	Liquid scintillation counter Wallac
Extracellular enzyme activity	Fluorescence-based, MUF and AMC – substrates	Hoppe (1993), Wittman et al. (2000)	Microtiter plate fluorometer Wallac 1420
CLPP*	Utilization of 95 carbon substrates	Laine et al. (1997)	Microplate reader interfaced to a Biolog Workstation (BIOLOG Inc., Hayward, USA)
Genetic structure of bacterial community	DGGE**	Muyzer et al. (1993), Nübel et al. (1996)	-

* Community-level physiological profiling

** Denaturing Gradient Gel Electrophoresis

3.3.2. Deposition rate and quality of periphyton in the lakes

Analysis for lake water and deposited material

Temperature and oxygen concentration in the littoral water were measured with a Yellow Springs Instruments (YSI) metre, and pH with a Mettler Delta 340 pH meter. Analyses described in the following list were made monthly for the littoral water of all three lakes (total five times).

- Chlorophyll a; ISO 10260 -standard modified into Finnish standard SFS 5772
- DOC concentration; measured with TOC-500 carbon analyser, Shimadzu
- Aromaticity of DOC compounds (fulvic acids); DOC-specific absorbance at 280 nm, Shimadzu 2401 PC UV/vis.
- Total amount of P and N; digestion to PO_4^{3-}-P and NO_3^--N using alkaline peroxosulphate oxidation, according to Koroleff (1979).
- Inorganic P and N, analysed as PO_4^{3-}-P, NO_2^- -N and NO_3^--N, NH_4^+-N according to Murphy and Riley (1962), Wood et al. (1967) and Solorzano (1969).

In June and July, the following parameters related to the chemistry of dissolved organic and inorganic compounds in littoral water of the three studied lakes were measured in order to describe differences in chemistry of DOM during development of the periphyton (III; Table 3).

- Molar absorption coefficient as absorbance in 436 nm (Reche and Pace, 2002).
- Fluorescence index (FI) of filtered lake water at emission wavelengths of 450 nm and 500 nm with an excitation wavelength of 370 nm (McKnight et al. 2001).
- Concentrations of iron (Fe), manganese (Mn), and magnesium (Mg) were assessed with atom absorption spectrophotometer.
- Light penetration (the photon flux density of 400−700 nm wavelengths) to the sediment surface and extinction coefficients (η) based on these values.

Total dry weight (dw) of the material settled in each littoral sampling location of the three lakes as well as the N and C contents of the dry matter were assessed. The more detailed descriptions of the methods and procedures used in all the analysis are given in the manuscript (III).

Sampling and analysis of the epiphytic biofilm

Periphyton growing on isoetid macrophytes *Lobelia dortmanna* (L.) in L. Ahvenlampi and L. Sammalisto, and *Isoëtes lacustris* (L.) in L. Kuorelampi was collected in July by diving. After primary treatment of the fresh epiphytic material, subsamples for the Chl *a* and phaeopigment analysis as well as the molecular genetic analysis of the procaryotic community composition were taken.

From the epiphytic material collected as pooled samples from several plants, stable carbon isotope ratio (δ13C reported in the conventional δ-notation with respect to the V-PDB carbonate standard analysis), chemical structure of the organic matter by using solid state 13C CP/MAS NMR and elemental analysis for the total amount of ions (P, K, Mg, Mn, Al and Cu) by using an ICP-MS, was determined in each lake.

For assessing the procaryotic community composition, DNA in the epiphyton samples was extracted and purified using the FastDNA® SPIN Kit for Soil following the manufacturer's protocol. Finally, the DNA samples were eluted to DNase/pyrogen-free water. The more detailed procedure for DGGE, sequencing and PCR of the eluted samples is described in the manuscript (III). Finally, PCR products were verified by agarose gel electrophoresis.

More detailed descriptions of the methods and procedures used in all the analysis mentioned above are given in the manuscript (III).

4. RESULTS AND DISCUSSION

4.1. Factors influencing quality of carbon pool in aquatic biofilms (II, III)

Quality of periphyton in the littoral area of three boreal lakes with dissimilar aquatic DOC concentration and aromaticity was assessed during the growing season (III). When the quality of carbon compounds in the water column and periphyton as well as several physical and chemical factors in these habitats were analyzed, some clear correlations were observed between the parameters measured. Significant (P < 0.001) positive correlations occurred between nearly all the factors related to the presence of allochthonous carbon compounds in lake water (DOC concentration, molar absorption coefficient, abs. 280 nm and fluorescence index FI) and in periphyton (proportion of aromatic functional groups and $\delta^{13}C$ ‰, III; Table 6). The proportion of aromatic functional groups in the periphyton was highest in the most humic L. Kuorelampi, also when compared to that of L. Sammalisto with a similar concentration but lower aromaticity of DOC. Periphyton collected from L. Ahvenlampi was lower in aromatic functional groups and higher in O-alkyl containing functional groups than those in the L. Sammalisto and L. Kuorelampi. In addition, the carbon isotopic ratio was about four units higher in L. Ahvenlampi than in the two more humic lakes. These results suggest that the quality of carbon pool in epiphyton was influenced by terrestrial, aromatic carbon compounds, which attach all the periphyton in humic lakes. They also indicate that long-term changes in the quality of terrestrial surface runoff and in the quality of aquatic DOC are reflected to the quality of periphytic carbon substrate pool in the littoral areas.

Similarly to the allochthonous carbon substrates, also autochthonous carbon pool in the epiphyton varied between the lakes with different qualities and quantities of DOC in the lake water. Total biomass and Chl *a* content of epiphyton correlated positively with DOC concentration as well as with the parameters related to the presence of humic substances (HS) in lake water (III; Table 6). This finding is consistent with some previous results, which show that the presence of aquatic dissolved organic matter enhances biomass formation and Chl *a* content of periphyton (Vinebrooke and Leavitt, 1998; Frost et al. 2007). This has been related to the attenuation of harmful UV radiation by dark-colored HS and more favorable nutrient conditions for algal growth. One potential reason for the differences in epiphytic biomass can

also be that grazing pressure caused by e.g. invertebrates and protozoa using periphyton as a food source differs between clear-water and humic lakes. In my study lakes, periphytic grazing activity was not assessed, but there is no evidence of higher grazing rates and consequently, lower biomass of periphyton in clear-water lakes as compared to humic lakes. However, autochthonous nutrient resources, i.e. biofilms and seston, have been found to make a significant contribution of energy transferred to higher trophic levels in brown-water lakes (Lau et al. 2014).

My results show that between clear-water and humic lakes and even between two humic lakes with similar DOC and TP concentrations but different quality of DOC, the nutrient content of periphyton may vary significantly. In the periphyton of L. Kuorelampi, phosphorus (P) concentration was higher and C:N:P -ratio was lower than in periphyton of the other two lakes (Table 3, III; Table 5). For that, the enhanced P binding ability of periphyton as induced by accumulated HS and the functional groups they consist is probably one reason. Furthermore, it has been earlier assessed, that the P content of nutrition is reflected to the P content of invertebrates in benthic habitats (Cross et al. 2003) and that biofilms are an important food source for shredding invertebrates in brown-water lakes (Lau et al. 2014). Hence, thick and phosphorus rich periphyton in the most humic lake Kuorelampi obviously makes a more significant pathway of nutrients and energy to invertebrates and consequently to higher secondary consumers as compared to L. Ahvenlampi and L. Sammalisto. This is also supported by Vesterinen and colleagues (2017), who assessed that in highly-humic lakes epiphyton growing on littoral *Sphagnum* moss beds had high productivity and through grazing, a strong contribution on the whole lake energy metabolism.

I suggest that in humic lakes the primary reason for the higher periphytic algal biomass is enhanced availability of nutrients, owing to the microbial decomposition of complex C substrates inside the periphytic EPS-matrix. Thus, differences in the periphytic nutritional conditions of the study lakes, for instance the higher P content in the periphyton of L. Kuorelampi, may have led to enhanced growth of epiphytic algae assessed in L. Kuorelampi as compared to two other lakes. According to my results, in lakes with different DOC concentration and quality, the potential periphytic P resources are higher in the most humic lakes, as the C:N:P ratios were lower there than in the clearwater lake. The significance of enzymatic decomposition of a newly deposited material after the terrestrial DOC addition was

obvious also in the mesocosm experiment, as humus addition into the water column caused changes in the carbon metabolism of sediment surface microbial communities. In the humic lakes, where phosphorus is usually the limiting nutrient, enzymatic recycling of P inside the thick periphyton can be assumed to be an important source of nutrients both to procaryotes and eukaryotic algae. As particulate and dissolved compounds adsorb abiotically to the mucilaginous periphyton, also humic compounds obviously participate to the nutrient pool utilized by periphytic organisms. However, also the central role of aquatic macrophytes as a substratum and nutrient source for periphytic communities must be taken into account when assessing the importance of different nutrient sources in littoral ecosystems.

Table 3. Quantitative and qualitative parameters of epiphytic material in the three studied lakes

	Ahvenlampi	Sammalisto	Kuorelampi
Biomass mg cm^{-2} (dw)	1.0 (0.5)	4.8 (0.1)	3.6 (2.1)
Chl a (mg g^{-1})	1.3 (0.9)	2.9 (0.4)	5.7 (0.9)
C%	32.6 (2.0)	29.3 (3.1)	33.2 (0.4)
N%	3.2 (0.3)	2.4 (0.2)	2.6 (0.2)
C/N	10.4 (0.3)	12.2 (0.5)	12.6 (0.8)
$\delta^{13}C$ ‰	−25.20	−29.02	−29.53
C : N : P	374: 31: 1	362: 36: 1	208: 16: 1
Functional groups %			
Aromatic	3.66	8.83	9.68
Carbonyl	2.22	2.76	2.56
O-alkyl	72.03	67.54	60.15
Alkyl	22.1	20.88	27.61

Deposition rate of organic material in littoral area of the three studied lakes did not correlate with DOC concentration or allochthony of carbon compounds in littoral water (III: Fig. 2). In

Swedish boreal lakes with different humic contents, flocculation and sedimentation of allochthonous DOC was assessed to be an important pathway for lacustrine carbon sequestration, and sedimentation rate in the lakes correlated with aromaticity of DOC in water (von Wachenfeldt and Tranvik, 2008; von Wachenfeldt et al. 2008). In those lakes as well as in several other studies sedimentation has been assessed in deep pelagial area, where stratification of the water column enables the gravitational settling of particles. In littoral area water is often in movement and continuous settling of particulate or colloidal material on the surfaces is therefore prevented. However, my results for the epiphyton of L. Sammalisto and L. Kuorelampi show that also in the littoral area humic substances accumulate to biofilms, but sedimentation/accumulation rates there are determined by different factors than in the lake pelagial areas.

My results show that the total amount of material and C deposited in the littoral area of the three studied lakes was obviously determined by resuspension. According to PCA, the amount of material deposited in the littoral area grouped together with oxygen and nitrate concentrations assessed from water column (III: Fig. 3). This can be explained by mixing of littoral water by wind, which causes resuspension of loose, amorphous material from the sediment surface to water column thus enhancing the deposition rates. Also, in earlier studies sediment resuspension and redeposition has been revealed to be a permanent feature in the littoral area (e.g. Bloesch and Uehlinger, 1986). Several factors, as exposure of littoral zone to wave action and water currents as well as presence of sheltering emergent macrophytes in shore area determine, how efficiently surface sediment is flushed to the water column and furthermore, transported to the pelagial area (Kairesalo and Matilainen, 1994).

During the growing season, deposition rates were mostly similar in all three lakes, but exceptionally in June, the deposition rate in L. Kuorelampi was about fourfold as compared to L. Ahvenlampi and sevenfold as compared to L. Sammalisto. During the experiment, there were no such changes in land-use of the drainage areas of the studied lakes, which could have caused e.g. high organic material load to L. Kuorelampi. The three lakes were also situated so close to each other that probably higher allochthonous material flow induced by e.g. drastic rain event in the drainage area could not explain the higher deposition rate assessed only in L. Kuorelampi. The most probable explanation for the higher deposition rate in L. Kuorelampi is the loose and fluffier structure of sediment surface material as compared to other two lakes

(field observation, not published). When proportions of carbon and nitrogen in the deposited material and littoral surface sediment as well as their C/N ratio were compared, it was possible to see some variation between the three lakes (III; Table 4). In L. Kuorelampi, all these variables were similar in the deposited material and surface sediment, whereas in the other two lakes especially the amount of N varied between the deposited material and surface sediment. Thus, it can be assumed that in L. Kuorelampi quality of material deposited in the littoral area was influenced more by resuspension from the sediment surface than in the other two lakes. As the total amount of deposited material in littoral area was obviously determined by wind, we can assume that under optimal wind conditions the loose sediment surface material mixed to the water column and resuspended efficiently causing the significantly higher deposition rate assessed in June in L. Kuorelampi as compared to two other lakes. However, more research is needed to address the reasons for drastic deposition peaks in littoral area and their influence on periphytic communities living there.

4.2. Changes in littoral sediment bacterial communities as induced by soil water pulse (I, II)

Both in mesocosms with humus (+ organic nutrient) addition and in mesocosms with inorganic nutrient amendment a similar increase in aquatic POC concentrations occurred during the first three experimental days (II; Fig. 5). Thereafter, the POC concentration behaved continuously in a similar manner, being 2.6 – 3.5 mg C L^{-1} after the first week and 1.0 mg C L^{-1} after day 22. Deposition rate was also similar in the two treatments. As primary production was high during the first 15 days of the experiment in both treatments and decreased after that, deposited material was obviously mostly consisted of algal material in both treatments. However, also more carbon-rich material must have been participated on the sedimentation during the experiment, as the C/N ratio of the deposited material in humus treatment varied between 11.2 and 13.2 being fairly high as compared to solely autochthonous material. For instance, in the pelagial area of a boreal lake with low allochthonous load deposited organic material had a C/N ratio of 6.9 – 10.1 (Koski-Vähälä et al. 2000). In the humus treatment, the C/N ratio of deposited material was slightly higher during the first two experimental weeks as compared to nutrient treatment, which obviously tells about the participation of humic carbon on the deposited material in the humus treated mesocosms (II; Fig 6 b.). To conclude, both in humus (+ nutrient) and inorganic nutrient treatments algal autochthonous material was mostly

responsible of the amount of deposited material, but in humus amended mesocosms carbon pool transported to the sediment consisted also of humic substances.

In sediments of the mesocosms, differences were found between the humus and inorganic-nutrient treatments in variety of bacterial metabolic attributes (paper II). Sediment β-glucosidase activity increased significantly in deep-water part of the humic-water mesocosms as compared to the nutrient amended mesocosms (Ptr < 0.05, Ptr and time < 0.001). In the shallow-water sediments, aminopeptidase activity was higher in the humus-treated mesocosms than in the nutrient mesocosms (P_{tr} < 0.02). The potential carbon substrate utilization efficiency (assessed as CLPP) in sediment was significantly higher in the nutrient treatment than in the humus-treated sediment both after 22 and 50 experimental days. Additionally, in those days the utilization of carbon substrates in the sediment samples grouped in PCA according to the humus treatment on the first principal axes (55% of the variation explained). This grouping was explained by a shift towards more effective utilization of small organic acids and sugars in humus treatment (results not shown). On the second principal axes sediments were grouped both according to the treatment ($p<0.01$) and time ($p<0.01$).

According to these results, terrestrial carbon amendment into the water column induced changes in the bacterial parameters tracing decomposition of freshly deposited carbon compounds in sediment, as the enhanced β-glucosidase activity, and more effective utilization of the small organic acids. On the contrary to these metabolic parameters, changes in the sediment bacterial cell abundance and biomass production were similar in the two treatments during the whole experiment. As sedimentation of autochthonous material was the main common factor changing in both treatments during the experiment, I hypothesize that autochthonous, labile organic material caused this similar development of the sediment bacterial cell numbers and biomass production in both treatments. This is supported by earlier studies, which have shown that respiration and biomass production correlate with the presence of autochthonous, nutrient rich material for instance in the water column of humic lakes (Kritzberg et al. 2005) and stream biofilms (Battin et al. 1999). Thus, material deposited on the sediment after soil water pulse assumingly influences sediment microbial communities both through low- and high molecular weight compounds. Impacts of the deposited compounds can also be negative; my results show that the humus amendment acted as an

inhibiting agent by decreasing potential carbon substrate utilization efficiency of sediment bacteria during the experiment. This inhibition caused by e.g. phenolic compounds has been assessed to be a complex reaction where metabolism of different bacterial species is suppressed under different DOC concentrations (McNamara and Leff, 2004).

When I assessed, how the presence of aquatic macrophytes influences the response of sediment bacterial communities on the soil water pulse, I found different bacterial response in rhizosphere and in plain (non-vegetated) sediment of the same depth (paper I). Bacterial communities in the plain sediment (2–5 cm depth) responded faster on the substrate addition into the water column than bacteria living in the rhizosphere sediment; after 22 days, humus addition had both positive and negative effects on bacterial communities of the plain sediment and effects were not seen in the rhizosphere sediment. For instance, number of bacteria was significantly higher and potential carbon substrate utilization efficiency of the bacterial community was lower in the plain sediment than in the rhizosphere sediment in both humus and inorganic nutrient treatments. After 50 days, the potential carbon substrate utilization efficiency, and also the number of carbon substrates used was significantly higher in the inorganic nutrient treatment than in the humic treatment, but the presence of *Lobelia* had no influence on the level of bacterial response anymore. Thus, the presence of *Lobelia* caused a delay in the sediment bacterial response on deposited material and this feature remained several weeks after the deposition event. Also, extracellular phosphatase activity developed differently between the rhizosphere and non-vegetated sediment after the deposition event. In the rhizosphere, bacteria showed higher phosphatase activity in the humus treatment than in the inorganic nutrient treatment, but in the non-vegetated sediment such reaction was not found. Hence, it is obvious that this phenomenon was induced by the different oxygen conditions and availability of phosphorus near roots of the macrophytes as compared to the non-vegetated sediment.

My results concerning the rhizosphere/ non-vegetated sediment show, that in the littoral area the complexity of environment, in this case the presence of aquatic macrophytes, creates a variety of habitats, where microbial reactions on terrestrial carbon and soil water amendment can vary via multiple ways. In the vicinity of roots, isoetid aquatic macrophytes create microhabitats, where oxygen conditions and nutrient availability are different as compared to the non-vegetated sediments. In earlier studies, presence of isoetid macrophytes has been

found to enhance the metabolic parameters of bacteria in surrounding sediments (e.g. Karjalainen et al. 2001). Although I didn't assess the BCC of these microhabitats, I assume that different environmental conditions between the rhizosphere and non-vegetated sediments form different species composition of bacterial communities and, consequently, a different reaction on substrate addition occurs. Recently, Pang and colleagues (2016) found an obvious difference in BCC of the rhizosphere and non-vegetated sediments in shallow littoral area, which supports my hypothesis.

Results of the mesocosm experiment show, that a drastic humic carbon pulse caused no phylogenetic shifts in sediment microbial communities whereas both positive and negative effects on metabolic attributes occurred. In streams, changes in quality and quantity of DOC and POC substrates have been assessed to affect the phylogenetic structure and function of streambed bacteria even in much lower DOM concentrations than those we used in our experiment (Battin et al. 2001, Findlay et al. 2003). Compared to those observations in the hyporheic microbial communities, we were able to measure a weaker response after the humus extract addition. This gives evidence that in littoral area the microbial communities of sediment surface are buffered against major changes in function or community composition after the deposition of recalcitrant organic material. Generally, microbes grown in biofilms have high resistance against metabolic changes when the carbon substrate supply is changed (Freeman and Lock, 1995). Our results also suggest that functional changes can occur in sediment surface communities without comparable phylogenetic responses after variation in carbon supply. This is opposite to findings of Smooth and Findlay (2001) and MacGregor et al. (2001), who found seasonal changes in the quality of deposited material cause both functional and structural shifts in microbial communities of the underlying sediment. It is obvious, that interactions between substrate conditions, BCC and metabolic changes in bacterial communities are complex, and vary as related to the specific environmental conditions and habitats, which also Judd et al. (2006) stated.

4.3. Influence of long-term and short-term load of humic material on the composition of periphytic aquatic microbial communities (II, III)

In the most humic L. Kuorelampi, phylotypes of *Proteobacteria* (e.g. γ-*Proteobacteria*) were prevalent in epiphytic biofilms. These phylotypes were closely related to eubacteria in soil,

delta, and estuarine environments. Some earlier studies have shown that planktic *Proteobacteria* are common in humic fresh waters and that they are even able to degrade humic substances (Eiler et al. 2003, Burkert et al. 2003). Ligi et al. (2014) assessed several phylotypes of *Proteobacteria* to be the most common bacterial phylogenetic group in wetland and Gao et al. (2005) found γ-*Proteobacteria* to be abundant in benthic communities exposed to high DOC load in stream environment. In estuarine water, γ-*Proteobacteria* was found to be well represented both in microbial community using HMW compounds and community using LMW compounds (Covert and Moran, 2001). However, also the substrata of the periphyton might influence the species composition of the periphytic microbial community, as in some studies, α-Proteobacteria and γ-Proteobacteria has been assessed to dominate especially in epiphytic biofilms (Tujula et al. 2010; Pang et al. 2016).

Microbial adaptation to utilization of different carbon substrates in natural periphyton is complicated to assess, as periphytic biofilms contain both algal-derived, autochthonous polysaccharides and carbon substances of allochthonous origin. Presence of certain phylogenetic microbial groups in periphyton cannot be used as a marker of utilization of solely autochthonous or allochthonous carbon substrates. However, my results and the earlier findings presented in the previous paragraph induce that the presence of group *Proteobacteria* may be attributed to long-term allochthonous carbon enrichment in epiphyton. For instance, in the clear-water L. Ahvenlampi members of this group were not found in periphyton. Some bacterial phylotypes found in this experiment were common to humic lakes L. Kuorelampi and L. Sammalisto, but in L. Kuorelampi certain *Proteobacteria*-phylotypes typical only to that lake were found. These bacterial phylotypes have earlier been found from soil, stream and sediment habitats with terrestrial influence, which supports the hypothesis that the periphytic microbial community in L. Kuorelampi is to some degree genetically adapted to environmental conditions where high molecular weight, terrestrial carbon compounds are available.

My results give evidence that the qualitatively and quantitatively different long-term carbon load between the lake ecosystems produce variation in the periphytic microbial community structure. I was able to assess a strong positive correlation between quality of carbon compounds, especially the presence of aromatic carbon, in water and periphyton, and, in the humic lakes, such kind of features in the periphytic BCC which can be attributed to humic environments. By contrast, in the boreal lakes with different allochthonous and autochthonous

carbon loads, Steger and colleagues (2011) found a strong positive correlation between microbial biomass in the littoral sediment and availability of autochthonous carbon in the water column. They also revealed that the microbial community composition in sediments (PLFA profiles) varied more as related to the season, than between the lakes with different carbon loads. This suggests that the allochthonous carbon load to lakes does not determine microbial parameters as BCC in sediment. I assessed the periphytic BCC only during the mid-summer stratification period, and BCC and aquatic carbon parameters were not compared between the seasons. It is possible, that in addition to the long-term quality of DOC in littoral water, also seasonal variation in the quality of aquatic carbon compounds have an influence on periphytic carbon quality and BCC in the littoral area of humic lakes.

In the water column of the mesocosms, humus pulse caused transient change in the microbial community composition as terrestrial microbes appeared along with the humus extract addition and disappeared during the experiment (Haukka et al. 2005). However, as no similar phylogenetic shift occurred in the sediment of humus treatment during the experiment, it is obvious that these terrestrial microbes did not settle into the sediment along with the deposited material. This is consistent with an earlier finding that flocs formed after terrestrial DOC addition into the lake water contain only minor proportion of bacteria (von Wachenfeldt et al. 2009). It has been earlier assessed that flocs formed in the water column contain bacteria and in some aquatic environments, particle-bound bacteria are even relatively numerous (Bell and Albright, 1982). However, this interaction between flocculated organic material and aquatic bacteria doesn't seem to occur when terrestrial bacteria are concerned. This gives evidence, that terrestrial bacteria which enter the lake along with surface runoff from drainage area do not contribute the sediment bacterial community but disappear after short time in the water column.

As stated in the previous chapter, similar changes in cell abundance and biomass production of sediment surface bacterial communities occurred both in humic-water and inorganic nutrient added mesocosms during the experiment. Also changes in bacterial community composition (BCC) during the experiment were similar in both treatments, whereas in some metabolic parameters some differences occurred. In the mesocosm experiment, a similar bacterial inoculum resulted in a similar BCC in situation where different substrates were offered to sediment bacterial communities. This agrees with some earlier studies, which have suggested

that changing environmental conditions do not determine BCC of heterotrophic microbes as strongly as the original community structure (e.g. Langenheder et al. 2006). For that, presence of generalist bacterial species in sediment may be the reason (Langenheder et al. 2005). Such bacterial species are e.g. able to utilize a wide range of carbon substrates and, consequently, dominate the BCC over more specialized bacterial phylogenetic groups in sediment. One reason for the similar development of BCC in the two treatments can also be the determining role of autochthonous material over allochthonous material in the sediment of both treatments, which supports the development of microbial communities adapted to the utilization of low molecular weight, labile substrates.

5. CONCLUSIONS

In my thesis, I was able to trace different factors, which have a role in carbon pool development of littoral periphyton and which, consequently, have an influence on structure and function of periphytic microbial communities as well. In lakes with naturally different concentration and quality of aquatic DOC, both allochthonous- and autochthonous-origin periphytic carbon pools as well as nutrient contents of the periphytic material varied. In the lake more susceptible to allochthonous carbon load, the proportion of aromatic carbon compounds was higher and carbon-nutrient ratio was lower in periphyton as compared to the clear-water lake. Aromatic carbon compounds obviously accumulate into the periphyton also through deposition and resuspension, in addition to direct adsorption. Periphytic algal biomass and, therefore, labile autochthonous carbon content was also higher in the more humic lakes as compared to the clear-water lake. It is evident, that mechanisms behind the variation in quality of periphytic carbon pool are determined by several physical, chemical, and biological factors in concert. Therefore, when the climate change induced shifts in aquatic DOC concentration and in the function of lake littoral area are evaluated, it is essential to take several abiotic and biotic factors into account simultaneously.

To conclude, terrestrial DOC flow from the drainage area to lakes seems to influence periphyton in the littoral area through different mechanisms depending on the duration of the carbon pulse. Drastic soil water pulse into the artificial lake littoral caused both short-term enhancing and suppressing metabolic impacts to the microbial communities of surface

sediment, but no shift in the community composition occurred. In the lakes with permanently different quality and quantity of DOC load, variations in the quality of periphytic carbon pool and microbial community composition were found. Thus, it seems that a more permanent change in allochthonous carbon load is required for shifting the periphytic microbial community structure in littoral area. In littoral area, living environment is also fragmented, which cause variation in microbial response on external impacts in community level. Presence of aquatic macrophytes had a clear impact on sediment microbial response on terrestrial-derived deposition event, probably as they create microhabitats with different oxygen and nutrient conditions as compared to plain sediments.

My results give evidence that the increased long-term DOC flow into lakes and changes in the quality of DOC shift the periphytic microbial community structure. However, the information my data offer is limited, and conclusions based on it should be drawn cautiously. It is evident, that shifting genetic structure of the periphytic microbial community leads to changes in the utilization and cycling of carbon and other nutrients inside the periphyton as well as between periphyton and surrounding water. This alters transfer of periphytic energy and material to higher trophic levels, which influences energy balance of the whole lake ecosystem. As littoral area comprehends a substantial number of different habitats which are influenced by complicated physical and chemical factors, further studies are needed to assess the interactive effects of increased aquatic DOC concentration and periphytic carbon pool on the structure and function of periphytic microbial communities.

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